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Nitric oxide-mediated anxiolytic-like and antidepressant-like effects in animal models of anxiety and depression

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Abstract

The effects of microinjection of the nitric oxide (NO) precursor L-arginine (L-Arg), the NO synthase (NOS) inhibitors *N*-methyl-L-arginine (L-NAME) and 7-nitroindazole (7-NI), and the cyclic guanosine 3',5'-monophosphate (cGMP) analog 8-Br-cGMP into the dorsal raphe nucleus (DRN) were assessed in rats using the elevated plus maze (EPM) and the forced swim test (FST). L-Arg (100 and 200 nmol) produced an anxiolytic-like effect in the EPM. 8-Br-cGMP (25 and 50 nmol) dose-dependently increased locomotor activity. In the FST, antidepressant-like effects were produced by L-Arg (50 and 100 nmol) and 8-Br-cGMP (12.5 and 25 nmol). Dual effects were observed with NOS inhibitors L-NAME and 7-NI in both the EPM and FST. While low doses of L-NAME (25 nmol) or 7-NI (1 nmol) induced a selective increase in EPM open arm exploration and a decrease in immobility time in the FST, high doses (L-NAME 400 nmol, 7-NI 10 nmol) decreased locomotor activity. These results show that interference with NO-mediated neurotransmission in the DRN induced significant and complex motor and emotional effects. Further studies are needed to elucidate the mechanisms involved in these effects.

Keywords: Dorsal raphe nucleus; Nitric oxide; Elevated plus maze; Forced swim test

1. Introduction

Midbrain raphe nuclei, including the dorsal raphe (DRN) and the median raphe nucleus (MRN), are proposed to modulate a variety of brain functions. These nuclei are the main origin for ascending and partially overlapping serotonergic projections (Lowry, 2002; Abrams et al., 2004) and have been considered to be an important component of the brain circuit that mediates anxiety- and depression-related behaviors (Jacobs and Azmitia, 1992; Graeff et al., 1996; Abrams et al., 2004). The role of serotonin (5-HT) in these disorders, however, is still unclear, with several apparently contradictory results. For example, activation of the DRN has been shown to reduce activity in the dorsolateral periaquequeductal gray (Lovick, 1994) and increase neural activity in the amygdala (Gonzalez et al., 1996), two brain areas proposed to mediated defensive responses. In addition, the DRN has been proposed to play a critical role in the development of learned helplessness, an animal model of depression (Maier and Watkins, 2005).

A large proportion of DRN 5-HT-positive neurons also contains neuronal nitric oxide synthase (nNOS) (Onstott et al., 1993; Dun et al., 1994; Wang et al., 1995; Xu and Hökfelt, 1997; Tagliaferro et al., 2001; Simpson et al., 2003), the enzyme responsible for nitric oxide (NO) production in the brain (Garthwaite et al., 1989). NO is synthesized from L-arginine (L-Arg) by nNOS in response to Ca^{+} influx induced by activation of *N*-methyl-D-aspartate (NMDA) glutamate receptors (Prast and Philippu, 1992). The physiological actions of NO are mainly mediated by stimulating soluble guanylate cyclase (sGC), which in turn leads to an increase in levels of cyclic guanosine 3'5'-monophosphate (cGMP) (Schuman and Madison, 1991). The resulting increase in cGMP levels then can modulate activity of cGMP-dependent protein kinases, cyclic adenosine monophosphate (cAMP)-phosphodiesterases, or ion channels (Garthwaite and Boulton, 1995).

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The presence of nNOS in many DRN neurons points to a significant involvement of NO in DRN function. NO produced in the DRN regulates sleep cycles (Monti et al., 1999), impairs escape performance, and enhances conditioned fear in rats exposed to an uncontrollable stressor (Grahn et al., 2000). Moreover, double-staining experiments using nicotinamide adenine dinucleotide phosphate diaphorase (NADPHd) histochemistry and *c-fos* immunohistochemistry in rats exposed to a cat or to the elevated plus maze (EPM) showed increased activation of NO-producing neurons in the DRN (Guimarães et al., 2005). Together, these results suggest that NO-mediated neurotransmission in the DRN could modulate stress responses.

NOS inhibitors have been reported to induce significant anxiolytic-and antidepressant-like effects in rodents. Systemic administration of the NOS inhibitor *N*-methyl-L-arginine ester (L-NAME) (Faria et al., 1997) or the more selective nNOS inhibitor 7-nitroindazole (7-NI) (Volke et al., 1997) produces anxiolytic-like effects in the EPM and decreases ultrasonic vocalization of rat pups (Campbell et al., 1999). The same compounds (Jefferys and Funder, 1996; Harkin et al., 1999; Yildiz et al., 2000), as well as the soluble guanylyl cyclases (sGC) inhibitors methylene blue (Eroglu and Caglayan, 1997) and 1H-(1,2,4)-oxidiazolo (4,3-1)quinoxalin-1-one (ODQ) (Heiberg et al., 2002), have been found to dose-dependently reduce immobility time in the forced swim test (FST). Interestingly, the antidepressant-like effects of NOS inhibitors may be dependent on endogenous 5-HT. Low and ineffective doses of L-NAME were able to potentiate the behavioral effects of imipramine and fluoxetine but not reboxetine, a noradrenaline reuptake inhibitor, in the FST (Harkin et al., 2003, 2004). The brain sites of these effects, however, are still unclear.

The objective of the present study, therefore, was to investigate if direct interference of NO-mediated neurotransmission in the DRN could modify the rat behavior in the EPM (an animal model of anxiety) and the FST (an animal model of depression).

2. Materials and methods

2.1. Animals

Three-hundred twenty-seven male Wistar rats weighing 280–310 g from the State University were used. These animals were transported to a colony room adjacent to the test laboratory 48 h before surgery. They were housed in groups of five per cage under a 12 h/12 h light/dark cycle (lights on at 07:00 h) at 23±1 °C and given free access to food and water. Procedures were conducted in accordance with the Brazilian Society of Neuroscience and Behavior Guidelines for the Care and Use of Laboratory Animals which comply with international laws. All efforts were made to minimize animal suffering.

2.2. Drugs

L-Arginine (L-Arg; 100, 200, and 400 nmol), NG-nitro-L-arginine methyl ester (L-NAME; 25, 100, 200, and 400 nmol) and 8-bromo-cyclic guanosine monophosphate (8-Br-cGMP; 6.25, 12.5, 25, and 50 nmol) were dissolved in sterile isotonic saline. 7-nitroindazole (7-NI; 1, 5, and 10 nmol) was dissolved in saline containing 2% dimethyl sulfoxide (DMSO) (vehicle). All drugs were purchased from Sigma Chemicals (St. Louis, MO, USA), and the solutions were prepared immediately before use. The doses were chosen based on previous studies that investigated the effects of these compounds in other brain structures such as the dorsolateral periaqueductal gray matter and medial amygdala (Guimarães et al., 1994; Forestirero et al., 2006).

2.3. Surgery

Animals (n=8–10/group) were anesthetized with sodium pentobarbital (45 mg/kg, i.p.) and fixed in a stereotaxic frame. Stainless steel guide cannulae (0.7-mm, outer diameter) were implanted in the DRN accordingly to the following coordinates: anterior/posterior: 1.4 from lambda; lateral: 3.7 mm; depth 6.2 mm (Paxinos and Watson, 1997). The guide cannula was inserted into the right hemisphere of the brain at an angle of 36° to the vertical plane to avoid the saggital sinus. The tip of the guide cannula was positioned 1 mm above the DRN. After surgical procedures, animals were group-housed until behavioral testing.

2.4. Procedure

Six days postsurgery, the animals were transported to the test laboratory and left undisturbed for at least 1 h prior testing. They were randomly assigned to one of the treatment groups.

For the microinjection procedure, animals were placed in individual cages and gently wrapped in a cloth. A thin dental needle (0.3 mm, outer diameter) was introduced through the guide cannula, and a volume of 0.3 µl was injected over 30 s using a microsyringe (Hamilton, USA) controlled by an infusion pump (Kd Scientific, Holliston, MA, USA). A PE10 polyethylene catheter was interposed between the upper end of each dental needle and the microsyringe, and the displacement of an air bubble inside the polyethylene tubing was used to monitor the microinjection procedure. The needle was held in place for an additional 1 min to maximize diffusion away from the injector tip.

For the combined treatments, animals first received a microinjection of either saline or L-NAME followed 10 min later by a second microinjection of saline or L-Arg. Following microinjections, the animals were left in their individual cages until being submitted to the EPM or FST.

The experiments were carried out in a sound-attenuated, temperature-controlled (23±1 °C) room illuminated by fluorescent bulbs. All behaviors were videotaped for later analysis.

2.5. The elevated plus maze

The EPM testing procedure was as described by Pellow and File (1986). The equipment was made of wood and had four arms of equal dimensions (50 cm×12 cm). Two of the arms were enclosed by 40-cm high walls and were arranged perpendicularly to two opposite open arms. The apparatus was elevated 50 cm

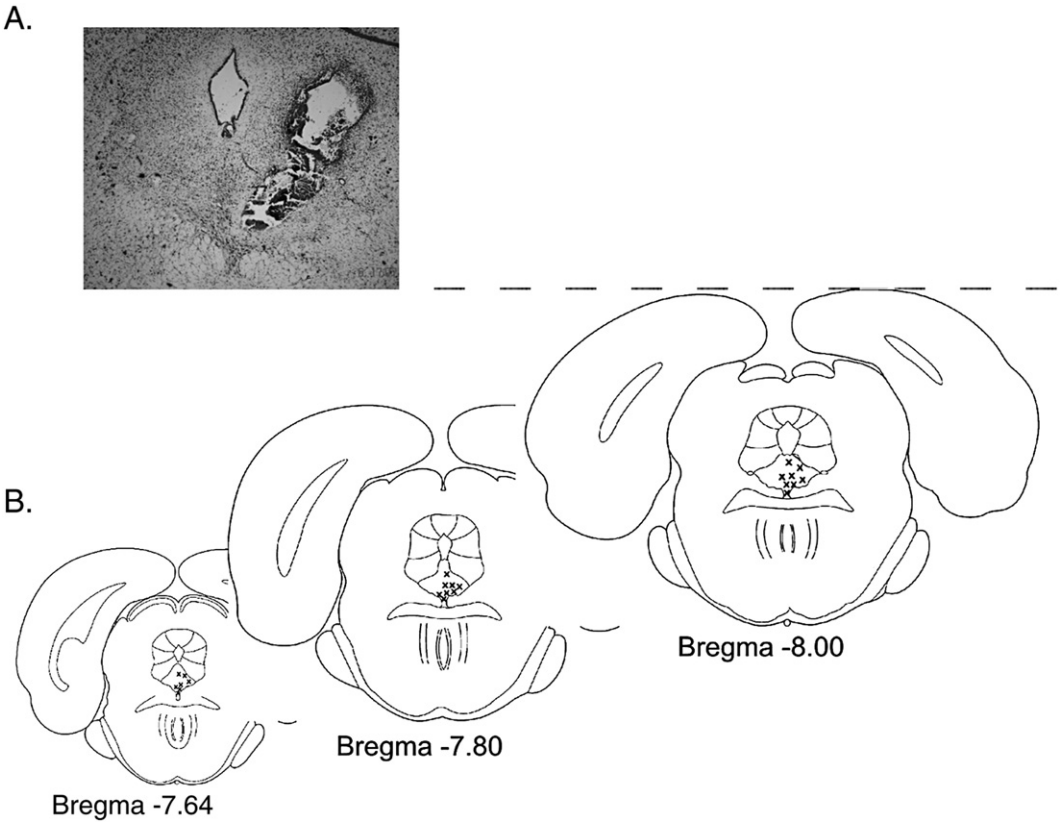

Fig. 1. Schematic representation of microinjection sites in the dorsal raphe nucleus (DRN) of rats. (A) Photomicrography of an injection site in the DRN. (B) Coronal sections showing the localization of microinjection sites into the DRN of rats. Numbers in the sections indicate the distance from bregma (Paxinos and Watson, 1997).

above the floor. To prevent falls, the open arms were surrounded by a Plexiglas rim 1 cm high.

10 min after the microinjections, rats were placed in the center of the maze facing a closed arm. The number of entries and time spent in open and closed arms of the maze were recorded for 5 min. The maze was cleaned with an alcohol solution after each trial.

2.6. Forced swim test

The procedures for the FST were similar to those first described by Porsolt et al. (1977). Initially, animals were place individually in a cylindrical tank (polyvinylchloride, 20 × 40 cm) containing clean water at 25 °C (25 cm deep) for 15 min (pre-test). 24 h later, the animals received the microinjections and after 10 min were submitted to a 5 min forced swim test session. During this session, the total amount of time in which the animals remained immobile (except for small limb movements necessary for floating) and the latency to the first episode of immobility were recorded.

After the swim sessions, the animals were taken out of the water and allowed to dry under a lamp (40 W for 15 min) before returning to their home cages.

2.7. Histology

After the behavioral tests, the rats were sacrificed under deep urethane anesthesia and were perfused through the left ventricle of the heart with isotonic saline followed by a 10% formalin solution. A dental needle then was inserted through the guide cannula and 0.2 μl of fast green was injected. The brains were removed and immersed in a 10% formalin solution for a minimum period of 3 days. 50 μm coronal brain sections then were obtained using a Cryostat (Cryocut 1800) and stained with cresyl violet. The injection sites were identified from diagrams from the atlas of Paxinos and Watson (1997).

2.8. Statistical analysis

The percentage of open arm entries (100 × open/total entries) and of time spent in the open arms (100 × open / open + enclosed) of the EPM were calculated for each animal. Data were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey test for multiple comparisons for each drug treatment. The significance level was set at $p < 0.05$.

3. Results

The representative localizations of microinjection sites are shown in Fig. 1.

3.1. Elevated plus maze

3.1.1. Anxiolytic-like effects of NO precursor L-arginine in the EPM

The dose-response curve for L-Arg (100–400 nmol) in the EPM is shown in Fig. 2. At the doses of 100 and 200 nmol, L-

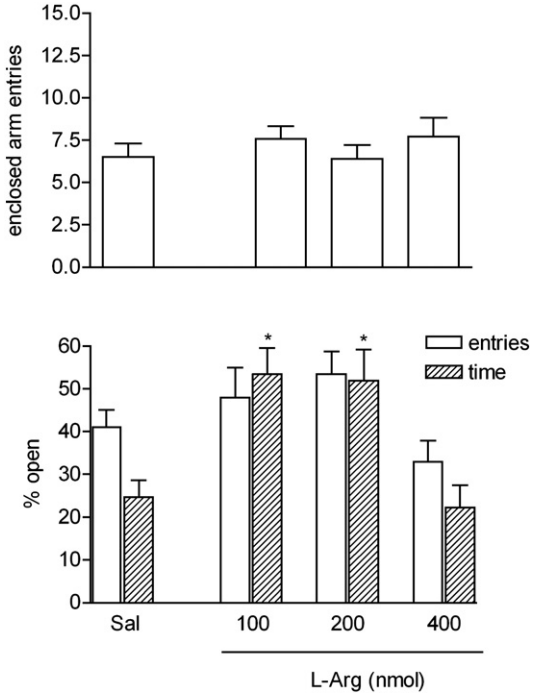

Fig. 2. Effects of the nitric oxide (NO) precursor L-argnine (L-Arg) (100–400 nmol) microinjected into the DRN of rats submitted to the elevated plus maze (EPM) for 5 min. The treatments were administered 10 min prior to testing. The columns represent the means, and the bars represent the SEM (n=9–10/group). **p<0.001 and *p<0.05 compared to the saline (Sal) group (ANOVA followed by the Tukey test).

Arg microinjected into DRN significantly increased the percentage of time spent in the open arms compared to the saline group ($F_{3,37}$=6.6, p<0.001). However, no significant effect was found in the percentage of open arm entries or in the number of closed arm entries (ANOVA, p>0.05).

3.1.2. 8-Br-cGMP, a cGMP analog, increased locomotor activity in the EPM

Rats injected with 8-Br-cGMP (25 and 50 nmol) into the DRN showed a significant increase in closed arm entries ($F_{3,38}$=22.9; p<0.001). No significant effect was observed concerning the percentage of entries or time spent in the open arms (ANOVA, p>0.05; Fig. 3).

3.1.3. The effects of NOS inhibitors L-NAME and 7-NI in the EPM

A low dose of L-NAME (25 nmol) increased the percentage of time spent in the open arms compared to the saline group ($F_{4,47}$=26.8, p<0.001). A higher dose of L-NAME (200 nmol) significantly decreased the percentage of entries ($F_{4,47}$=14.6, p<0.001) and time spent (p<0.001) in the open arms compared to the saline group (Fig. 4A), suggesting an anxiogenic-like effect. However, at dose of 400 nmol, L-NAME caused a significant decrease in the number of closed arm entries ($F_{4,47}$=9.2, p<0.001).

Low doses of 7-NI (1 and 5 nmol) significantly increased the percentage of entries ($F_{3,31}$=8.4, p<0.001) and time spent ($F_{3,31}$=39.6, p<0.001) in the open arms compared to vehicle (Fig. 4B). However, at 10 nmol, 7-NI caused a significant decrease in all parameters evaluated in the EPM, including closed arm entries ($F_{3,31}$=22.8, p<0.001).

3.1.4. L-Arginine counteracted the effect of L-NAME in the EPM

As shown in Fig. 5, L-Arg (100 nmol), which alone did not modify EPM exploration, reversed the decrease in the percentage of entries ($F_{3,31}$=5.7, p<0.05) and time spent

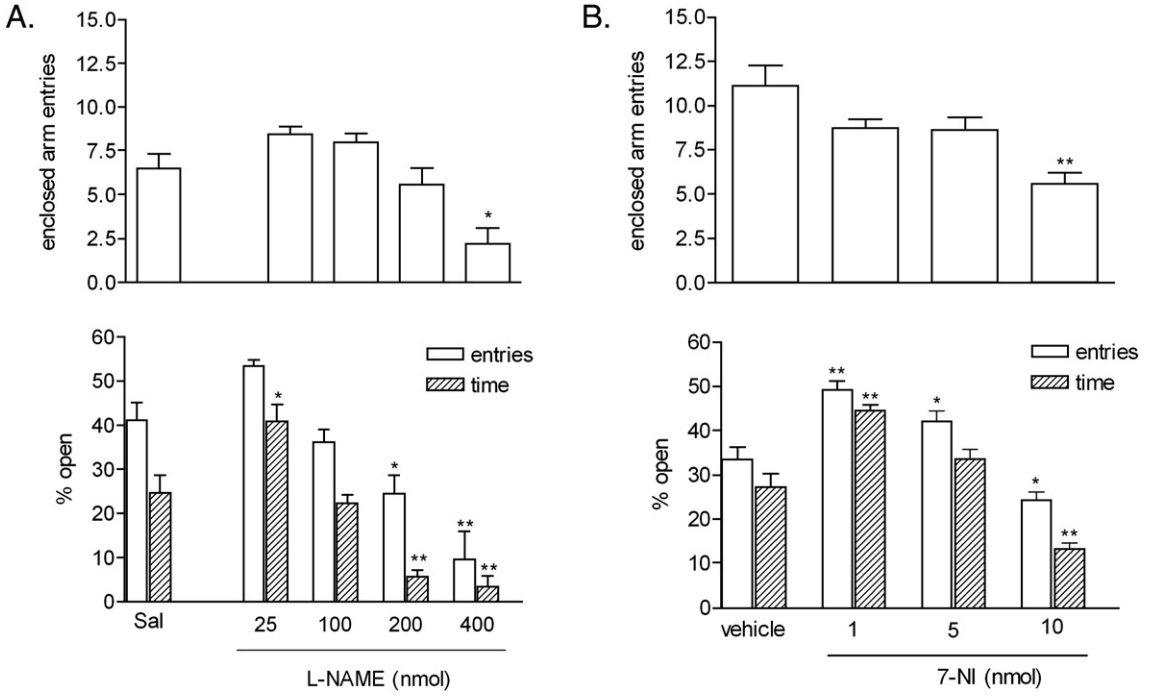

Fig. 3. Effects of 8-Br-cGMP (12.5–50 nmol), an analog of cyclic guanosine 3',5'-monophosphate (cGMP), microinjected into the DRN of rats submitted to the EPM for 5 min. The treatments were administered 10 min prior to testing. The columns represent the means, and the bars represent the SEM (n=9–10/group). **p<0.001 compared to the saline (Sal) group (ANOVA followed by the Tukey test).

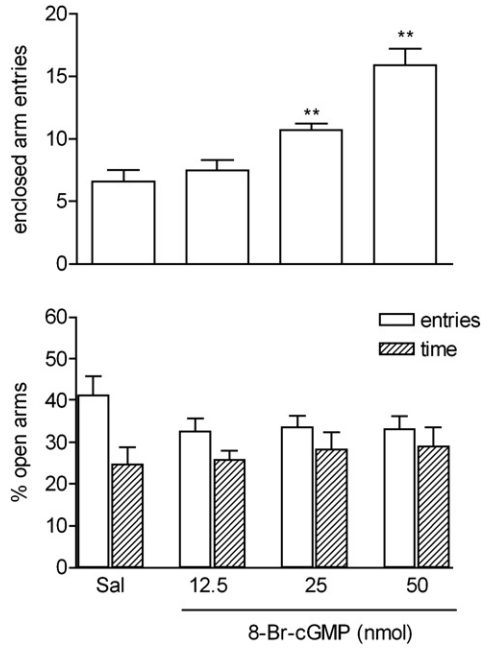

Fig. 4. Effect of nitric oxide synthase (NOS) inhibitors *N*-methyl-L-arginine (L-NAME) (25–400 nmol) and 7-nitroindazole (7-NI) (1–10 nmol) injected into the dorsal raphe nucleus (DRN) of rats submitted to the EPM for 5 min. The treatments were administered 10 min prior to testing. The columns represent the means, and the bars represent the SEM (n=7–8/group). **p<0.001 and *p<0.05 compared to saline (Sal) (panel A) or vehicle (2% DMSO, panel B) (ANOVA followed by the Tukey test).

($F_{3,31}$=3.1, p=0.04) in the open arms of the EPM induced by L-NAME (200 nmol) compared to the control group. It also reversed the decrease in the number of closed arm entries ($F_{3,31}$=8.4, p<0.001) caused by the NOS inhibitor L-NAME.

3.2. Forced swim test

3.2.1. L-Arginine and 8-Br-cGMP decreased immobility time in the FST

A significant decrease in immobility time in the FST was observed in animals that received L-Arg (100 nmol; $F_{3,33}$=23.2, p<0.001) or 8-Br-cGMP (12.5 and 25 nmol; $F_{3,33}$=47.6, p<0.001) into the DRN (Table 1). A concomitant increase in the latency to the first episode of immobility also was observed with both compounds ($F_{3,33}$=11.6, p<0.001).

3.2.2. The effects of NOS inhibitors L-NAME and 7-NI in the FST

L-NAME at 25 nmol injected into the DRN significantly decreased immobility time in the FST ($F_{4,41}$=15.2, p<0.001), suggesting an antidepressant-like effect. However, at a high dose, L-NAME produced the opposite effect, increasing immobility time (200 nmol; $F_{4,41}$=22.3, p<0.001) with a concomitant decrease in latency (L-NAME 100 and 200 nmol; $F_{4,41}$=16.9, p<0.001) compared to the saline group (Table 1).

Similar effects were found for 7-NI (Table 1). At a low dose (1 nmol), the drug significantly decreased immobility time ($F_{3,31}$=41.1, p<0.001) and increased latency ($F_{3,31}$=11.6, p<0.001), while at a higher dose (10 nmol) it produced an opposite effect, increasing immobility time and decreasing latency.

3.2.3. The antagonistic effect of L-NAME on the effect of L-arginine in the FST

Considering the results from the combined treatment shown in Table 2, pretreatment with L-NAME at a subeffective dose of 50 nmol into the DRN attenuated the effect of L-Arg (100 nmol) on immobility time ($F_{3,31}$=16.1, p<0.001) and latency ($F_{3,31}$=18.3, p<0.001).

4. Discussion

The EPM has been widely used as a model that provides independent measures of anxiety-like behavior (percentage of entries or time spent on the open arms) and locomotor activity (number of closed arm entries) in rodents (File, 2001; Carobrez and Bertoglio, 2005). Animals exhibiting anxiety-like behavior naturally avoid the open arms of the EPM, and anxiolytic compounds typically increase open arm exploration without affecting the number of closed arm entries (Carobrez and Bertoglio, 2005). In the present study, direct injection of L-Arg (100 and 200 nmol) into the DRN significantly increased the percentage of time spent on the open arms of the EPM, suggesting that facilitation of NO-mediated neurotransmission in the DRN causes an anxiolytic-like effect. Corroborating this hypothesis, local injection of L-NAME 200 nmol, induced anxiogenic-like effects—an effect that was prevented by injection of L-Arg. L-Arg

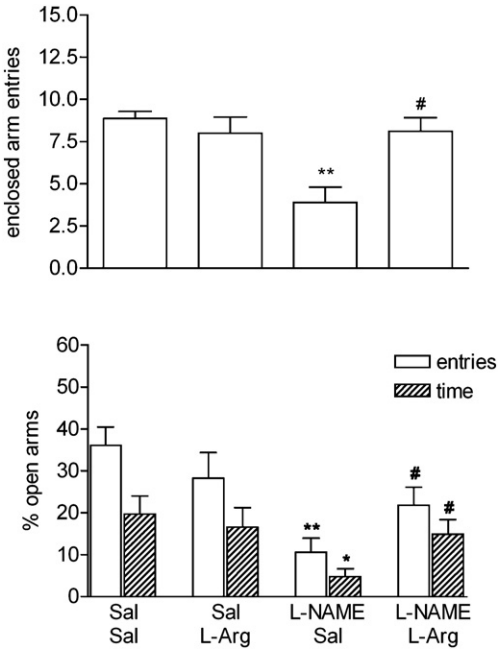

Fig. 5. L-Arginine (L-Arg) (100 nmol) counteracted the effect of *N*-methyl-L-arginine (L-NAME) (200 nmol) in the elevated plus maze. Animals first received a microinjection of saline (Sal, 0.3 μl) or L-NAME followed by a second microinjection of either Sal or L-Arg 10 min later. The animals were submitted to the elevated plus maze for 5 min. The columns represent the means, and the bars represent the SEM (n=7–8/group). **p<0.001 and *p<0.05 compared to Sal+Sal, and $^{\#}p$<0.05 compared to L-NAME+L-Arg groups (ANOVA followed by the Tukey test).

Table 1
Effects of L-Arg, L-NAME, 7-NI and 8-Br-cGMP on rat behavior in the forced swim test

Treatment	Immobility time (s)	Latency (s)
Saline (0,3 μl)	201.1±6.2	38.8±7.0
L-Arg (nmol)		
25	198.4±6.1	39.9±4.0
50	154.6±12.2*	62.3±7.2
100	110.9±10.3**	95.9±11.3**
L-NAME (nmol)		
25	159.6±8.1*	51.1±4.3
50	189.1±8.2	29.4±4.0
100	227.1±10.6	14.3±1.8*
200	256.6±4.3**	2.8±1.2**
8-Br-cGMP (nmol)		
6.5	166.3±12.4	57.4±5.2
12.5	107.4±10.2**	69.8±8.2
25	47.2±9.2**	191.8±17.9**
Vehicle (0,3 μl)	176.1±4.2	45.1±3.9
7-NI (nmol)		
1	136.3±6.9^{++}	70.1±8.2^{+}
5	192.5.±7.1	35.1±3.1
10	228.1±5.1^{+}	31.1±3.7

Drugs were administered (0,3 μl/30 s) into DRN 10 min before the test session in. Data presented are mean values±SEM (n=8–10/group). *p<0.05 and **p<0.001 compared to saline; +p<0.05 and ++p<0.001 compared to vehicle (2% DMSO) (ANOVA followed by the Tukey test).

failed to modify EPM exploratory activity, perhaps reflecting a stress influence of two intracerebral injections. However, the effects of L-Arg and the NOS inhibitors were limited. While in low doses L-NAME and 7-NI caused anxiolytic-like effects, high doses of the same compounds produced a decrease on locomotor activity. Moreover, intra-DRN injection of the cGMP analogue 8-Br-cGMP (25 and 50 nmol) increased the number of closed arm entries but failed to produce any change in anxiety indices measured in the EPM.

The FST is a widely used experimental model for the detection of antidepressant-like effects in rats and mice (Porsolt et al., 1977). Exposure to a pre-test swim session reduces the latency to immobility and lengthens the time spent in this posture in a subsequent test session. Antidepressant agents consistently reverse the effects of the previous swimming stress on the test session (Porsolt et al., 1977; Lino de-Oliveira et al., 2005). In the present study, low doses of L-NAME and 7-NI produced antidepressant-like effects, while high doses increased immobility time. Corroborating the latter finding, intra-DRN injections of L-Arg (50 nmol) or 8-Br-cGMP (12.5 and 25 nmol) resulted in antidepressant-like effects, the former being prevented by a non-effective dose of L-NAME. The present results, therefore, suggest that disruption of NO-mediated neurotransmission in the DRN produces complex effects in animals tested on the EPM and FST.

Dual effects following interference with NO-mediated neurotransmission have been widely reported in several behavioral tests (Cappendijk et al., 1995; Leza et al., 1996; Czech et al., 2003; Masood et al., 2003, Del Bel et al., 2005), including animal models of anxiety and depression. Systemic or intra-dorsolateral periaqueductal gray matter or medial amygdala injection of NOS inhibitors, for example, produced anxiolytic- (Guimarães et al., 1994; Faria et al., 1997; Volke et al., 1997, 2003a; Forestiero et al., 2006) or anxiogenic-like (Quock and Nguyen, 1992; De Oliveira et al., 1997; Monzon et al., 2001) effects in rodents submitted to the EPM (for review, see De Oliveira et al., 2001). Contradictory findings also have been reported in the FST (Da Silva et al., 2000; Inan et al., 2004; Kaster et al., 2005; Érgün and Ergün, 2007). Although several groups have shown that L-Arg does not induce depressive-like behaviors in rodents (Yildiz et al., 2000; Heiderg et al., 2002; Volke et al., 2003b), it can produce opposite effects in the FST depending on the dose used (Inan et al., 2004). Considering local injection, Joca and Guimarães (2006) showed that 3-morpholinosydnonimine (SIN-1), a NO donor, injected into the hippocampus of rats did not produce any behavioral change in the FST. Antidepressant-like effects, however, were obtained with administration of the NOS inhibitor 7-NI into the same region. Only one study has investigated the effects of a NOS inhibitor injected directly into the DRN in an animal model of depression. Using the learned helplessness model, Grahn et al. (2000) showed that L-NAME (18 nmol) reversed escape failures induced by a previous exposure to inescapable tailshock when administered before the escapable test session. The present study corroborates this result. 25 nmol of L-NAME was able to decrease immobility time in the FST without changing locomotor activity in the EPM. In contrast to this antidepressive-like effect, L-Arg and 8-Br-cGMP also decreased immobility. The latter compound, however, increased locomotor activity in the EPM, an effect that may have interfered with the FST results. Thus, the effects observed with NO-interfering compounds into the DRN may reflect a general interference with locomotor activity of the animals.

Several reasons could account for the complex effects of NO-related compounds in behavioral tests. NO effects may vary depending on the functional state of the target neurons and the instant composition of the extracellular fluid (Contestabile, 2000). At high concentrations, NOS inhibitors may be converted to L-Arg (Hecker et al., 1990) or interfere with endothelial NO synthase (Esplugues, 2002). Moreover, although NO usually facilitates neurotransmitter release, it can have biphasic effects (Segieth et al., 1995). For example, low doses of the NO donor *S*-nitroso-*N*-acetylpenicillamine (SNAP) facilitated dopamine release, while a high dose elicited a long-

Table 2
Effects of pretreatment with L-NAME 50 nmol on the behavioral action of L-Arg 100 nmol in the FST

Treatment	Immobility time (s)	Latency (s)
Saline+saline	179.9±10.9	37.8±2.3
Saline+l-Arg 100 nmol	121.9±6.8**	71.4±5.8**
L-NAME 50 nmol+saline	190.9±5.2	33.8±3.3
L-NAME 50 nmol+L-Arg 100 nmol	172.5±6.3$^{\#}$	40.0±3.9$^{\#}$

L-NAME 50 nmol and L-Arg 100 nmol were administered (0,3 μl/30 s) into the DRN 20 and 10 min prior to the test session, respectively. Data presented are mean values±SEM of the groups (n=8/group). **p<0.001 compared to Saline+saline group and $^{\#}p$<0.05 compared to Saline+L-Arg 100 nmol groups (ANOVA followed by the Tukey test).

lasting reduction in basal dopamine levels (Segieth et al., 2000). Biphasic effects of L-Arg on striatal dopamine efflux also have been reported (Silva et al., 1997). Moreover, low concentrations of NO have been shown to stimulate, and higher concentrations to inhibit, dopamine uptake by synaptosomes (Chaparro-Huerta et al., 1997). These effects may explain why, in contrast to several studies showing cataleptic effect of NOS inhibitors (see Del Bel et al., 2005, for review), Dall'Igna et al. (2001) reported a cataleptic effect of a higher dose of sodium nitroprusside, a NO donor.

Nonspecific interference of NO-related drugs in locomotor activity has been reported in various studies using NOS inhibitors (Volke et al., 1995; Eroglu and Çaglayan, 1997). These drugs reduce spontaneous locomotor activity (Sandi et al., 1995; Dzoljic et al., 1997; Del Bel et al., 2002) and hyperlocomotion induced by phencyclidine (Noda et al., 1995) and induce catalepsy in rodents (Del Bel et al., 2005). Moreover, nNOS mutant mice have altered locomotor abilities (Kriegsfeld et al., 1999), and NOS inhibitors cause a loss of motor coordination (Harkin et al., 1999). Although these effects seem to depend on direct effects of NO on striatal dopaminergic neurotransmission (Del Bel et al., 2005), the present results suggest that the DRN also could be involved. The DRN sends 5-HT fibers to the striatum, a motor-related area (Lowry et al., 2005) where they can modulate motor function (Bishop et al., 2004).

In addition to the striatum, the DRN also innervates emotion-related areas such as the amygdala and the dorsolateral periaqueductal gray (Lowry et al., 2005). 5-HT is proposed to play a dual role in these two structures, facilitating inhibitory avoidance in the former and inhibiting escape responses in the latter (for review, see Graeff et al., 1996). These effects may explain the conflicting results obtained in the EPM with 5-HT-related drugs (Griebel, 1995). According to this view, the EPM would be a mixed anxiety model, where animals display two different strategies of defense, namely avoidance of open arms and escape from an open arm to enter a safer, closed arm (Carobrez and Bertoglio, 2005). In the present study, L-Arg did not change the percentage of entries into the open arms but significantly increased the percentage of time spent on these arms. This suggests that L-Arg treatment could impair escape responses. A more intense locomotor effect produced by 8-Br-cGMP would have prevented a similar effect by this drug in the EPM. Another possibility is that L-Arg is acting through NO effects that do not involve cGMP synthesis such as interference with adenosine diphosphate ribosyltransferases (Garthwaite and Boulton, 1995) or direct activation of calcium-dependent potassium channels, changing the release of several neurotransmitters (Shin et al., 1997).

The activity of DRN neurons is under an intricate regulatory process mediated by the medial prefrontal cortex and involving excitatory glutamate inputs to serotonergic and γ-aminobutyric acid (GABA) neurons (Celada et al., 2001). The glutamatergic inputs are mediated, at least in part, by NMDA receptors, resulting in NO formation. NO could thus modulate the local release of glutamate, GABA, and 5-HT, resulting in complex effects on DRN neuronal activity and interference in motor and emotion-related processes.

In conclusion, the present results show that interference with NO-mediated neurotransmission in the DRN induced significant and complex motor and emotional effects. Further studies are needed to elucidate the mechanisms involved in these effects.

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